Journal of Chemical Ecology, Vol. 9, No. 9, 1983

IDENTIFICATION OF A FEMALE-PRODUCED SEX PHEROMONE FROM THE SOUTHERN CORN ROOTWORM, *Diabrotica undecimpunctata howardi* BARBER[1,2]

P.L. GUSS,[3] J.H. TUMLINSON,[4] P.E. SONNET,[4] and J.R. McLAUGHLIN[4]

[3]*USDA, ARS, Northern Grain Insects Research Laboratory*
Rural Route 3, Brookings, South Dakota 57006
[4]*USDA, ARS, Insect Attractants Behavior and Basic Biology Research Laboratory*
P.O. Box 14565, Gainesville, Florida 32604

(Received September 27, 1982; revised December 15, 1982)

Abstract—A sex pheromone has been isolated and identified from virgin females of the southern corn rootworm (SCR), *Diabrotica undecimpunctata howardi* Barber. The synthesized compound, 10-methyl-2-tridecanone was shown to be attractive to males of the SCR, and also to males of *D. u. undecimpunctata* Mannerheim, the western spotted cucumber beetle (WSCB), and of *D. u. duodecimnotata* in Mexico. Males of both the SCR and the WSCB strongly preferred the *R* over the *S* enantiomer. The resolved enantiomers were not tested against *D. u. duodecimnotata*.

Key Words—Coleoptera, Chrysomelidae, *Diabrotica*, southern corn rootworm, spotted cucumber beetle, western spotted cucumber beetle, sex pheromone, 10-methyl-2-tridecanone, ketone.

INTRODUCTION

The southern corn rootworm (SCR), *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae), is a widely distributed polyphagous insect of particular economic importance on peanuts, domestic cucurbits, and corn. Often called the spotted cucumber beetle, the SCR is

[1] Coleoptera: Chrysomelidae.
[2] Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.

included in a taxon containing two other subspecies; the western spotted cucumber beetle (WSCB), *D. u. undecimpunctata* Mannerheim, whose range is restricted to the far western United States and the upper Baja peninsula, and *D. u. duodecimnotata* Harold, which is found in Mexico (Smith, 1966).

Larvae of the SCR are most damaging to corn in the southeastern U.S. where those hatched from eggs of overwintering adults either feed on seedling corn roots or bore into the base of the stem (Arant, 1929). SCR larvae are also serious pests of peanuts, causing damage by penetrating the developing peanut—either consuming it or providing entry for pathogenic microorganisms (Campbell and Emery, 1967). In addition to corn and peanuts, larvae of the SCR are known to attack cucurbits and most legumes (Campbell and Emery, 1967).

Adults of the SCR have been collected from some 280 species of plants (Sell, 1916) but are of primary concern on cucurbits and peanuts. Adults of the WSCB have been known to cause severe damage to forage corps in the Pacific Northwest (Rockwood and Chamberlin, 1943). In addition to feeding damage, *D. undecimpunctata* spp. have been implicated as vectors of a number of plant diseases (see e.g., Doolittle and Beecher, 1950; Porter and Smith, 1974; Coudriet et la., 1979).

A sex pheromone has been demonstrated for the SCR, and Branson et al. (1978) showed that males of *D. u. duodecimnotata* in Mexico are attracted to traps baited with unfractionated volatiles from female SCR originating in South Dakota. This paper reports the isolation, identification, and synthesis of the major pheromonal component produced by female SCR.

METHODS AND MATERIALS

Pheromone Collection and Bioassay. Insects used in this study came from a laboratory colony reared according to Branson et al. (1975). Virgin females were isolated from males within 24 hr of emergence and held in screened cages (30 × 30 × 30 cm) for 3 days before being placed in a pheromone collection chamber. Adults were provided water and a dry diet mixed with a small amount of honey (Guss and Krysan, 1973).

For pheromone collection, about 15,000 virgin females were placed in a metal screened cage (29 × 29 × 89 cm) which was subsequently enclosed in a Plexiglas box (31 × 31 × 91 cm) containing a hole (35 mm diam) on either end. A glass tube (130 × 18 mm ID) filled with about 15 g of Porapak Q® for pheromone absorption (Byrne et al., 1975) was attached to one end of the Plexiglas box with a rubber stopper. The other end of the tube was attached to a vacuum source. The chamber was located in an isolation room at 24° C with a 12-hr photophase. Unpurified air at a rate of 6.5 liters/min was passed over the insects and through the Porapak Q filter. A complete exchange of air in the

chamber took about 13.5 min. Twice each week the apparatus was disassembled, and dead insects were removed and replaced with fresh females. Food and water were also changed at these times. Once each week the Porapak Q filter was removed and replaced with a fresh one. At this time the live insects were transferred to a clean screened cage, and the Plexiglas box was thoroughly cleaned before reassembly.

Laboratory bioassay techniques developed for the western corn rootworm (Guss et al., 1982) were directly adaptable to the SCR. Four or five male SCR beetles were placed in a disposable Petri dish (150 × 15 mm) and allowed to acclimate for 15 min. Then a test compound in 1–5 μl of hexane was applied to a filter paper chip (5 mm^2) and, after the solvent evaporated (about 10 min), the treated chip was placed in the Petri dish. Positive responses consisted of orientation of the beetles toward the chip, distinctive antennal waving, and copulatory behavior toward the other males.

Field bioassy of extracts and chromatographic functions during purification of the pheromone were carried out in a small domestic cucurbit patch (about 85 m^2) near Brookings, South Dakota. Because native populations were low, the patch was periodically infested with laboratory-reared SCR males. The traps consisted of 360-ml inverted drink cartons coated with Stickem Special®. Pheromone was dispensed from cotton wicks (30 × 10 mm diam) placed on top of the traps. Trap height was slightly above canopy height or about 0.4 m. This same type of trap was used for field testing synthetic pheromone against the WSCB near Riverside, California, and *D. u. duodecimnotata* in the state of Jalisco, Mexico; rubber septa were used as dispensers in those studies.

For field studies comparing natural and synthetic pheromone, sticky liners from Zoecon 1C® traps were used. Wooden dowels (0.63 cm diam) were run through the liner mounting holes so that the trap consisted of a vertical white cardboard panel with Stickem on one face. When the dowel was inserted in the ground, the top of the trap was just above the height of the vegetation. For each test the traps were placed in a randomized complete block and treatment positions were rerandomized each time traps were checked. Traps were about 3 m apart in each block, and blocks were 30–45 m apart. The tests were run in plots of mixed cucurbits (squash, melon, cucumber) in Gainesville, Florida, in June–July 1981. Pheromone was dissolved in hexane and appropriate quantities of each solution were pipetted onto a 4.5-cm-diam Whatman No. 1 filter paper disk just prior to baiting the traps. The filter paper bait was fastened just below the top edge of the sticky surface of the panel at ca. 3:20 to 4:30 PM each day.

A dose–response curve was obtained with traps baited as above with half of a rubber septum (A.H. Thomas No. 8753-D22, Philadelphia, Pennsylvania, 5 × 9 mm, split lengthwise and extracted for 1 hr with

methylene chloride) impregnated with 0.3–100 μg of synthetic pheromone in 25 μl of hexane. These traps were inspected and rerandomized within blocks each day.

Pheromone Purification. Pheromone was extracted from the Porapak Q of each filter with 50 ml ether–hexane (60:40) in a 125-ml Erlenmeyer flask. The mixture was allowed to steep with occasional agitation for 24 hr after which the Porapak Q was removed by filtration. The remaining solvent containing the pheromone was reduced in volume under vacuum at 0° C to about 1 ml, and the concentrated solution was subjected to gas–liquid chromatography (GLC) without further treatment.

Micropreparative GLC for isolation of the pheromone was performed with a Varian model 1400 gas chromatograph equipped with a flame ionization detector. Stainless-steel columns were packed with 30% DEGS on 60/80 mesh Chromosorb-W® (2 mm ID × 9.1 m) or 1.5% OV-101 on 100/120 mash Chromosorb G-HP® (2 mm ID × 1.5 m). The injection port temperatures were 185° C and 160° C with each column type, respectively; the detector temperature was 250° C. The DEGS and the OV-101 columns were maintained at 165° and 130° C, respectively. The carrier gas (N_2) flow rate through all columns was 20 ml/min.

The chromatograph was modified to accommodate a 90:10 effluent splitter and an external, dry ice-cooled fraction collector (Brownlee and Silverstein, 1968). Fractions were collected in 1.5 × 305-mm capillary tubes and were subsequently eluted with a minimal volume of hexane.

Synthesized pheromone was purified by preparative, high-pressure liquid chromatography (HPLC) on a 1.27-cm OD × 25-cm stainless-steel column packed with 5 μm Lichrosorb SI60® (Heath et al., 1978). A Lab Data Control Constametric II G® pump delivered the hexane–ether (97:3) mobile phase at 4 ml/min, and the eluting components were detected with a Waters model R401 differential refractometer.

Pheromone Analysis and Identification. Natural and synthesized pheromone was analyzed on 0.25 mm (ID) glass capillary columns prepared in the manner described by Heath et al. (1980). An SP2340® column, 60 m long, was maintained at 60° C for 2 min after injection, programed at 32°/min to 150° C, and then operated isothermally. An OV-101 column, 52 m long, was maintained at 50° C for 2 min after injection, programed at 32°/min to 170° C, and then operated isothermally. Both columns were operated in the splitless mode with an N_2 carrier gas flow of 9.5 cm/sec in a Hewlett-Packard model 5710A gas chromatograph equipped with a model of 18740B split–splitless injector system.

Mass spectral data were obtained with a Finnigan model 3200 mass spectrometer equipped with both chemical ionization (CI) and electron impact (EI) sources. A Varian model 1400 gas chromatograph equipped with

a 5% OV-1 column, 2.2 mm (ID) × 2 m, served to introduce samples to the CI source. Methane was employed as reagent gas and GLC carrier gas. The EI source was served by a Finnigan model 9500 gas chromatograph equipped with an OV-1 column of the same dimensions. Helium was normally used as the carrier gas.

Hydrogenolysis was carried out in the injector gas chromatograph that served the EI source by the method of Beroza and Sarmiento (1963, 1964). About 6 cm of a 3.2-mm-ID stainless-steel tube was filled with neutral Pd catalyst and placed in the injector port ahead of the OV-1 column. The catalyst was maintained at 260° C for the hydrogenolysis, and H_2 was used as the carrier gas at 30 ml/min.

For NMR, a 25-μg sample of the purified natural pheromone was chromatographed on a 1.2-m × 2-mm (ID) stainless-steel column packed with 10% Carbowax 20 M terephthalate on 120/140 mesh Chromosorb W operated at 150° C in a Varian model 1400 gas chromatograph equipped with a 95:5 effluent splitter and external fraction collector as described by Brownlee and Silverstein (1968). The pheromone was collected in a glass capillary cooled with solid CO_2 and then rinsed with 22 μl of benzene-D6 into an NMR tube, the top of which was 5 mm (OD), with a 50 × 2-mm (OD) coaxial extension on the bottom (Wilmad Glass Co., catalog No. 507 with WGS-5BL stem). This solution filled the 2-mm (OD) extension of the NMR tube to a height of 17 mm. The NMR spectrum was obtained with 500 scans (16 K data points, 5-μsec pulse, total experimental time 23.5 min) on a Nicolet® 300 MHz Fourier transform NMR spectrometer interfaced to a Nicolet 1280 data system.

RESULTS AND DISCUSSION

Hexane–ether extracts of Porapak Q filters from the collection chamber were tested for pheromone activity in the laboratory and in the field. In the laboratory bioassay, SCR males exhibited behavior typical of other *Diabrotica* spp. in the presence of their pheromones, i.e., orientation toward the pheromone source, distinctive antennal waving, and copulatory behavior toward other males. In the field, SCR males were captured on sticky traps baited with these same volatiles.

Laboratory bioassay of gas chromatographic fractions obtained from the Porapak-collected volatiles indicated that the pheromonal activity was confined to a single area of the chromatogram eluting after 42–46 min on DEGS. Fractionation of total volatiles, as well as rechromatography of the active area from DEGS, on OV-101 also produced a single area of activity eluting after 20–24 min. Field bioassay of these same fractions confirmed the above results. Comparison of the active peak with paraffin hydrocarbons

yielded a retention index of 2020 on DEGS and 1540 on OV-101 (Kovats, 1965).

Pheromone was purified for identification by fractionation of the Porapak extract on DEGS and subsequent refractionation of the eluate from the active area on OV-101. This produced a single, well-resolved peak of active material. Analysis of the purified pheromone from the packed OV-101 column on OV-101 and SP2340 capillary columns indicated that it was about 99% pure. About 60 μg of pure pheromone was obtained from about 50,000 virgin females over a 3-month period.

The methane CI mass spectrum (Figure 1A) established that the molecular weight of the pheromone was 212 with diagnostic peaks at *m/e* 211 (M − 1), 213 (M + 1), 241 (M + 29), and 253 (M + 41). The facts that the M + 1 peak is the base peak and that there is almost no fragmentation suggest a fairly stable parent molecule with an oxygen function. In the EI mass spectrum (Figure 1B), the base peak at *m/e* 43, the strong peak at *m/e* 58, and the weak peak at *m/e* 57, plus the moderately strong peak at *m/e* 71 strongly suggest a methyl ketone with no substitution on the carbons α or β to the carbonyl (Budzikiewicz et al., 1967).

A comparison of the EI mass spectra of authentic 2-tetradecanone with the natural pheromone at this point indicated that there were several similarities in the low mass region of the spectra but distinct differences in the high mass region. Additionally, the pheromone eluted prior to 2-tetradecanone on both capillary columns, suggesting the possibility of a branched-chain 14-carbon methyl ketone.

The NMR spectrum of the isolated pheromone (Figure 2) confirmed the presence of a methyl ketone with unsubstituted methylenes α and β to the carbonyl with the following signals: δ 1.93 [t, 2H, —(C=O)—$\underline{CH_2}$—CH_2], 1.64 [S, 3H, CH_3—(C=O)—], and 1.48 ppm [m, 2H, —(C=O)—CH_2—$\underline{CH_2}$—]. Additionally, two overlapping signals in the methyl region appear to be a triplet centered at δ 0.91 and a doublet centered at δ 0.90, indicative of $\underline{CH_3}$—CH_2— and $\underline{CH_3}$—CH—, respectively. Thus the spectroscopic data support a 14-carbon ketone with the following structure:

$$CH_3-\overset{\overset{O}{\|}}{C}-CH_2-CH_2(CH_2)_n-\overset{\overset{CH_3}{|}}{CH}-(CH_2)_mCH_2CH_3$$

Hydrogenolysis of the pheromone in the gas chromatographic injector leading to the EI source produced a product with the mass spectrum shown in Figure 3. The peak at *m/e* 198(M+) confirms the 14-carbon skeleton indicated by mass spectra of the parent compound. The peaks at *m/e* 70, 71, and 154, 155, are of greater intensity than would be expected in a normal hydrocarbon and represent preferred cleavage on either side of a methyl branch located at the 4-position in the chain. Thus, the structure of the

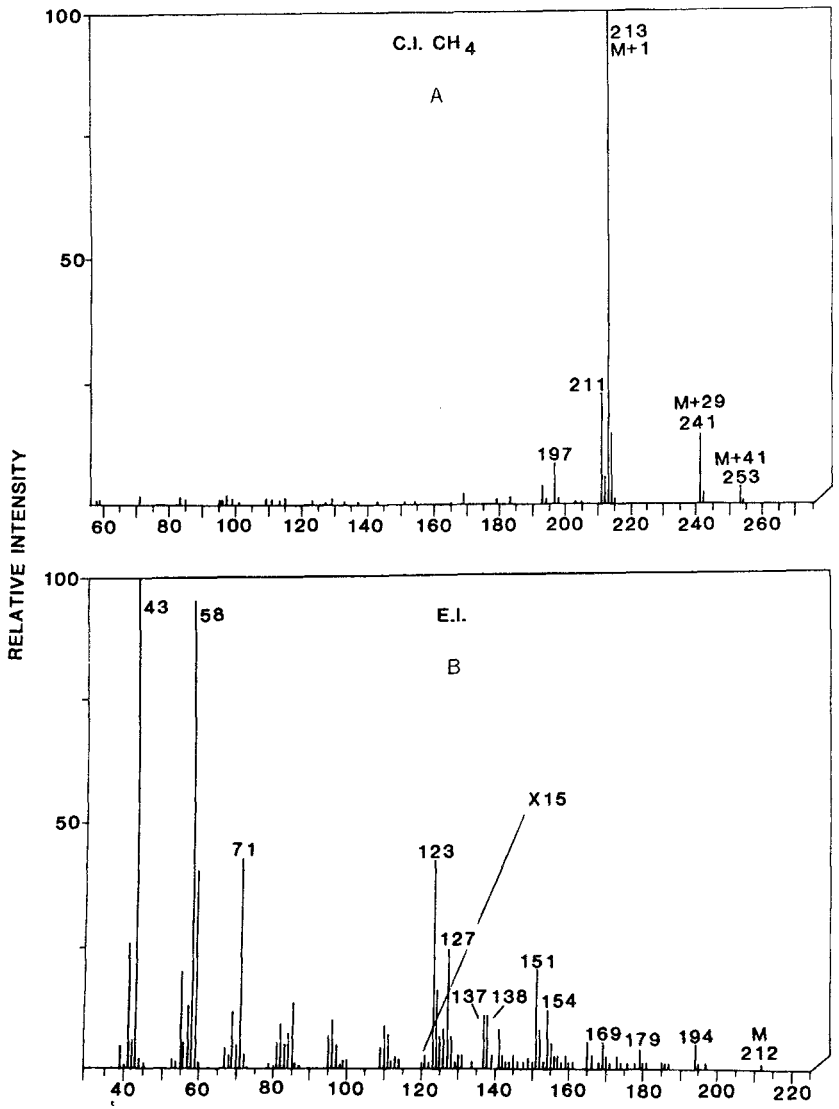

FIG. 1. (A) Chemical ionization (CH_4) and (B) electron impact mass spectra of *D. undecimpunctata* pheromone.

hydrogenolysis product is established as 4-methyl tridecane, and the pheromone of the southern corn rootworm is therefore 10-methyl 2-tridecanone (I, Figure 4).

Synthesis of I. The synthesis of racemic 10-methyl-2-tridecanone was initiated in a manner analogous to the preparation of the sex pheromone of

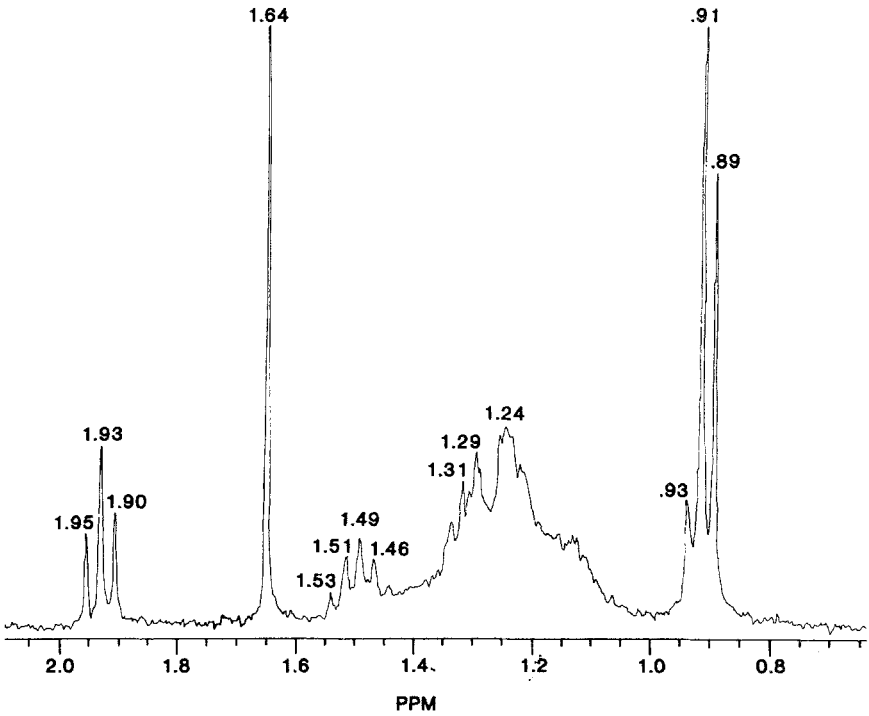

FIG. 2. 300-MHz proton magnetic resonance spectrum of *D. undecimpunctata* pheromone.

the western corn rootworm (Guss et al., 1982). Methyl cyclopropyl ketone (Figure 4) was allowed to react with *n*-propyl magnesium chloride. The intermediate tertiary carbinol was isomerized to the homoallylic bromide, II (bp 91–93° C at 27 mm, yield: 58%) using cold 48% hydrobromic acid. This reaction was first described by Julia (1961), and the halide II has been previously prepared in this fashion by Kulesza et al. (1969) who reported bp 51–52° C at 2 mm. Compound II was reduced to 1-bromo-4-methylheptane, III, by hydrogenation with platinum oxide in propionic acid. The alkyl bromide was obtained in ca. 91% crude yield; apparently hydrogenolysis of the C–Br bond under these conditions is insignificant [bp not determined because the halide foamed badly; NMR ($CDCl_3$, δ) 0.86 (t, $\underline{CH_3}CH_2$), 0.87 (d, $\underline{CH_3}CH$), and 3.40 ppm (m, 2H, $CH_2\underline{CH_2}Br$)]. Bromide III was converted first to a Grignard reagent and then to a cuprate with methyl copper (Bergbreiter and Whitesides, 1975). The organocuprate was coupled to the propionate ester of 6-iodo-2-hexanol, IV (the synthesis of this compound will be reported by us in connection with other research) to give the required carbon skeleton in the form of propionate ester V [IR (CCl_4) 1740 cm^{-1};

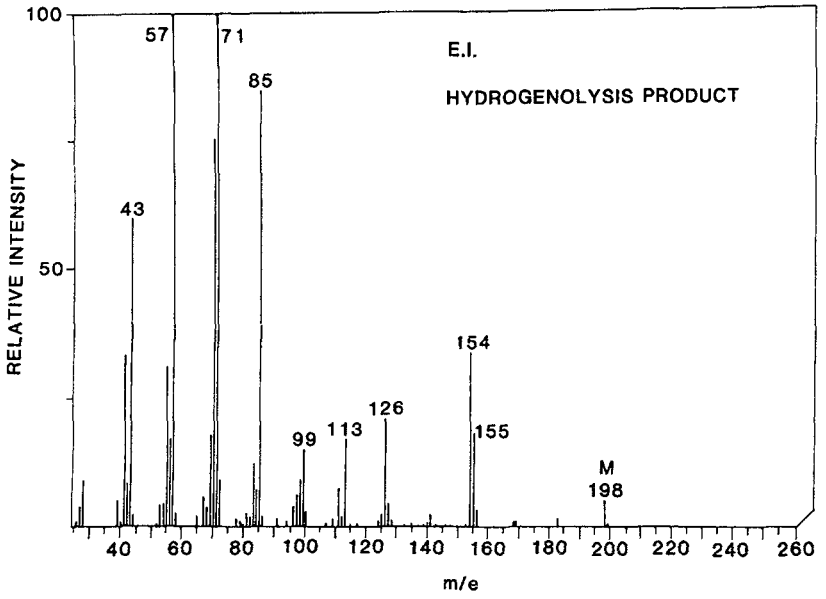

FIG. 3. Electron impact mass spectrum of product of hydrogenolysis of *D. undecimpunctata* pheromone.

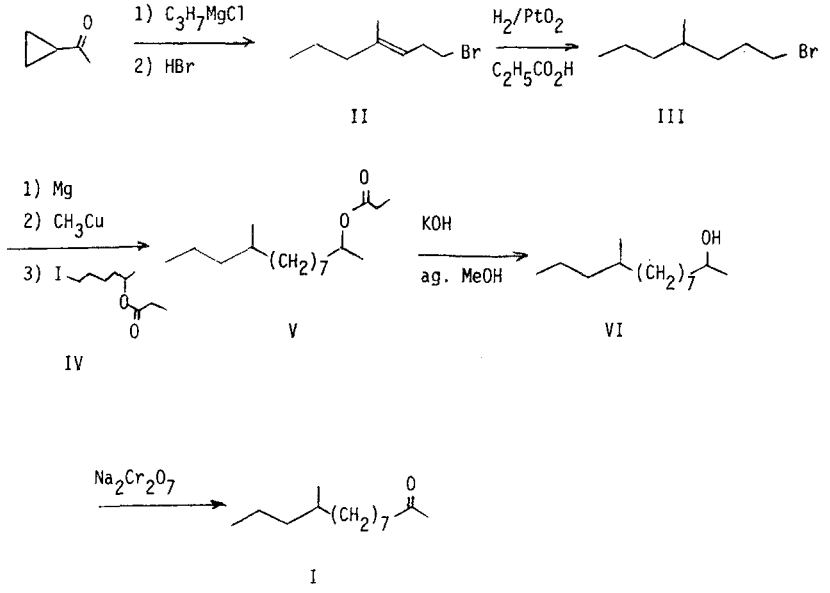

FIG. 4. Synthesis of racemic 10-methyl-2-tridecanone.

CIMS: $(M + 1)^+$ 271, $(M + 1—HO_2CC_2H_5)^+$ 197]. Saponification of V produced alcohol VI in 68% overall yield from III [IR (CCl_4) 3640 cm^{-1}; NMR ($CDCl_3$, δ) 0.85 (d, 3H, $J = 7$ Hz, $\underline{CH_3}CH$), 0.88 (t, 3H, $J = 7$ Hz, $\underline{CH_3}CH_2$), and 3.64 ppm (t, 2H, $J = 6$ Hz, $\underline{CH_2}OH$); CIMS: $(M)^+214$, $(M - 1)^+$ 213, $(M + 1\ 18)^+$ 197]. Oxidation of VI to I was accomplished with aqueous acidic dichromate in near quantitative yield [IR (CCl_4) 1720 cm^{-1}; NMR ($CDCl_3$) δ 2.13 (s, 3H, $\underline{CH_3}C{=}O$), 2.42 (t, 2H, $J = 7.4$ Hz, $CH_2\underline{CH_2}C{=}O$) ppm; CIMS: $(M + 1)^+$ 213].

The synthesized 10-methyl-2-tridecanone was purified by HPLC on Lichrosorb SI60. GLC analysis of the material collected from HPLC on the SP2340 and OV101 capillary columns indicated that it was greater than 99% pure. The pure natural pheromone and the synthesized 10-methyl-2-tridecanone cochromatographed on both capillary columns, and the EI and CI mass spectra of the synthesized compound were identical to the respective spectra of the natural pheromone.

Field Tests. The racemic synthetic 10-methyl-2-tridecanone (I) applied at 1 μg in 25 μl of hexane on filter paper was compared to natural, pure pheromone at the same rate and to a hexane blank. The test was run from June 13–15, 1981, with two randomized blocks. Traps baited with I captured a mean of 11.5 ± 1.8 (SE) males per trap per day while traps baited with natural pheromone captured 22.0 ± 3.9 (SE) males. These means are significantly different (ANOVA, $P \leqslant 0.01$). No males were captured in the hexane-baited traps (control). Thus, the racemic I appeared less attractive than the natural pheromone. Attraction of both natural and synthetic pheromones lasted only about 20 min with the filter paper formulation.

When adjusted for pheromone content, filter paper formulations of total volatiles collected on Porapak were equally attractive to males as those baited with purified natural pheromone (Table 1). The presence of another

TABLE 1. FIELD RESPONSES OF SCR MALES TO NATURAL SCR PHEROMONE AND 10-METHYL-2-TRIDECANONE (I) DISPENSED FROM FILTER PAPER, JULY, 10–13, 1981, GAINESVILLE, FLORIDA

Pheromone source	Amount[a] (μg)	$\bar{X} \pm SE^b$ males/trap/replicate
Unpurified volatiles	1	21.8 ± 1.5 a
	3	42.8 ± 5.1 b
Natural pure pheromone	1	22.4 ± 2.0 a
Synthetic racemic (I)	2	29.9 ± 4.2 a

[a] Amount of active material based on GLC analysis.
[b] Mean with a different letter is significantly different from the other means, NMR, $P \leqslant 0.01$. Randomized complete block (2) experiment ($N = 6$).

TABLE 2. FIELD RESPONSE[a] OF SCR MALES TO ENANTIOMERS OF 10-METHYL-2-TRIDECANONE DISPENSED (100 μg) FROM SPLIT 5 × 9-mm RUBBER SEPTUM, JULY 23–25, 1981, GAINESVILLE, FLORIDA

Synthetic material	$\bar{X} \pm$ SE[b] males/trap/replicate
R enantiomer (≤0.4% S)	73.5 ± 19.6 a
S enantiomer (≤0.1% R)	7.0 ± 2.3 b
1:1 $R + S$ (50 μg each)	53.9 ± 17.8 c
Racemic I	51.9 ± 12.6 c

[a] Randomized complete block experiment. One block in each of 3 widely separated peanut fields ($N = 9$).
[b] Means followed by different letters differ significantly, Duncan's NMR using 2-way ANOVA, $P \leq 0.05$.

pheromone component is therefore not indicated. Twice the dose of I was required to obtain attraction equal to the insect-produced material, suggesting that only one enantiomer of I is the active component. This contention is supported by results obtained with preparations of the separate enantiomers (Sonnet, 1982) evaporated from rubber septa. The R-enantiomer (Table 2) exhibited greater activity than I or a 1:1 mixture of $R + S$ enantiomiers. The S enantiomer had virtually no activity.

A dose–response effect was demonstrated when I was evaporated from rubber septa (Table 3). The data were best characterized by the equation $y = 0.57 + 1.83 \ln x$ ($R^2 = 0.95$) over a dose range of 1–100 μg.

The high degree of laboratory and field activity, dose–response effect, and differential activity of enantiomers leads us to the conclusion that (R)-10-

TABLE 3. DOSE-RESPONSE EFFECTS OF RACEMIC 10-METHYL-2-TRIDECANONE DISPENSED FROM RUBBER SEPTA ON TRAP CATCHES OF SCR MALES, MAY 28–JUNE 1, 1981, GAINESVILLE, FLORIDA

Dose (μg)	$\bar{X} \pm$ SE males/trap/replicate ($N = 12$)
100	8.7 ± 2.6
30	5.1 ± 1.5
10	2.8 ± 0.9
3	1.3 ± 0.7
1	0.1 ± 0.03
0.3	0 ± 0
Blank	0.1 ± 0.7

methyl-2-tridecanone is the major natural product produced by SCR females that is responsible for the attraction of males. The total sex pheromone system of the SCR may contain other components responsible for untested aspects of its mating behavior.

In addition to the SCR, 10-methyl-2-tridecanone (I) was tested for activity toward the other two known subspecies in this taxa. Near Riverside, California, wild males of the western spotted cucumber beetle, *D. u. undecimpunctata* Mannerheim (WSCB), were highly attracted to traps baited with 100 μg of racemic I on rubber septa. Subsequent tests indicated that the WSCB also strongly preferred the *R* over the *S* enantiomer (A.N. Kishaba, personal communication). Males of *D. u. duodecimnotata* also were captured in traps baited with racemic I in the state of Jalisco, Mexico (H. Valdés M. personal communication).

Acknowledgments—We thank Dr. A.N. Kishaba for field tests involving the WSCB and Ing. H. Valdés M. for field tests involving *D. u. duodecimnotata*. We also thank A.J. Proveaux for mass spectral analyses, J.R. Rocca for obtaining NMR spectra of the natural and synthetic pheromones, and M.A. Williams for technical assistance.

REFERENCES

ARANT, F.S. 1929. Biology and control of the southern corn rootworms. Ala. Agric. Exp. Sta. Bull. 230, 46 pp.

BERGBREITER, D.E., and WHITESIDES, G.M. 1975. Synthesis of fatty acids using organocopper(I) ate complexes derived from Grignard reagents. *J. Org. Chem.* 40:779–782.

BEROZA, M., and SARMIENTO, R. 1963. Determination of the carbon skeleton and other structural features of organic compounds by gas chromatography. *Anal. Chem.* 35:1353–1357.

BEROZA, M., and SARMIENTO, R. 1964. Carbon skeleton chromatography using hot-wire thermal-conductivity detection. *Anal. Chem.* 36:1744–1750.

BRANSON, T.F., GUSS, P.L., KRYSAN, J.L., and SUTTER, G.R. 1975. Corn Rootworms: Laboratory Rearing and Manipulation. USDA ARS-NC-28, 18 pp.

BRANSON, T.F., GUSS, P.L., and KRYSAN, J.L. 1978. Winter populations of some *Diabrotica* in central Mexico: Voltinism and pheromone response. *Ann. Entomol. Soc. Am.* 71:165–166.

BROWNLEE, R.G., and SILVERSTEIN, R.M. 1968. A micro-preparative gas chromatograph and a modified carbon skeleton determinator. *Anal. Chem.* 40:2077–2079.

BUDZIKIEWICZ, H., DJERASSI, C., and WILLIAMS, D.H. 1967. Mass Spectrometry of Organic Compounds. Holden-Day, San Francisco, pp. 134–138.

BYRNE, K.J., GORE, W.F., PEARCE, G.T., and SILVERSTEIN, R.M. 1975. Porapak Q collection of airborne organic compounds serving as models for insect pheromones. *J. Chem. Ecol.* 1:1–7.

CAMPBELL, W.V., and EMERY, D.A. 1967. Some environmental factors affecting feeding, oviposition, and survival of the southern corn rootworm. *J. Econ. Entomol.* 60:1675–1678.

COUDRIET, D.L., KISHABA, A.N., and CARROLL, J.E. 1979. Transmission of muskmelon necrotic spot virus in muskmelons by cucumber beetles. *J. Econ. Entomol.* 72:560–561.

DOOLITTLE, S.P., and BEECHER, F.S. 1950. Transmission of downy mildew of cucumber by the spotted cucumber beetle. *Phytopathology* 40:870–871.

GUSS, P.L., and KRYSAN, J.L. 1973. Maintenance of southern corn rootworm beetles on a dry diet. *J. Econ. Entomol.* 66:352–353.

GUSS, P.L., TUMLINSON, J.H., SONNET, P.E., and PROVEAUX, A.T. 1982. Identification of a female produced sex pheromone of the western corn rootworm. *J. Chem. Ecol.* 8:545–556.

HEATH, R.R., PROVEAUX, A.T., and TUMLINSON, J.H. 1978. A simple terminator for high efficiency liquid chromatography columns. *J. High Resol. Chromatogr. Chromatogr. Commun.* 1:317–319.

HEATH, R.R., BURNSED, G.E., TUMLINSON, J.H., and DOOLITTLE, R.E. 1980. Separation of a series of positional and geometrical isomers of olefinic aliphatic primary alcohols and acetates by capillary gas chromatography. *J. Chromatogr.* 189:199–208.

JULIA, M. 1961. Nouveaux alcools á chaine isoprenique et leur préparation. Fr. patent 1,213,486, July 31, 4 pp.

KOVATS, E. 1965. A retention index system. *Adv. Chromatogr.* 1:229–235.

KULESZA, J., GORA, J., and KOSESKA, K. 1969. Synthesis of β-elgenol and its analogs. *Riechst. Aromen Koerperpflegem.* 19(5):192–200.

PORTER, D.M., and SMITH, J.C. 1974. Fungal colonization of peanut fruit as related to southern corn rootworm injury. *Phytopathology* 64:249–250.

ROCKWOOD, L.P., and CHAMBERLIN, T.R. 1943. The western spotted cucumber beetle as a pest of forage corps in the Pacific Northwest. *J. Econ. Entomol.* 36:837–842.

SELL, R.A. 1916. Notes on the 12-spotted cucumber beetle. *J. Econ. Entomol.* 9:551–556.

SMITH, R.F. 1966. The distribution of diabroticites in western North America. *Bull. Entomol. Soc. Am.* 12:108–110.

SONNET, P.E. 1982. Syntheses of the stereoisomers of the sex pheromone of the southern corn rootworm and lesser tea tortrix. *J. Org. Chem.* 47:3793–3796.